IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Hammond et al.

Serial No: 09/866,557

Filed: May 24, 2001

For: Methods and Compositions for RNA Interference

Attorney Docket No. CSHL-P02-010

Art Unit: 1637

Examiner: Cynthia B. Wilder

Commissioner of Patents
Washington, D.C. 20231

Declaration Under 35 U.S.C. §1.132 of Gregory Hannon

Sir:

I, Gregory Hannon of Huntington, NY, hereby declare as follows:

1. I am a co-inventor of the abovementioned application (herein the "Hammond Application") which teaches and claims methods for attenuating expression of a target gene in mammalian cells by introducing into mammalian cells suspended in culture an expression vector encoding a hairpin RNA.

2. I have reviewed the outstanding Office Action dated April 21, 2005, (herein the "Office Action") that was issued in connection with the Hammond Application, and the pending claims of the Hammond Application. The pending claims cover methods for attenuating gene expression in mammalian cells suspended in culture using a transcribed hairpin RNA. I understand that the Examiner has rejected the pending claims on the grounds that the Hammond Application does not provide sufficient guidance on how to make and/or use transcribed hairpin RNAs in mammalian cells without undue experimentation. I respectfully disagree with the Examiner's grounds for these rejections.

3. Since the filing of the Hammond Application and publication by my lab of articles corresponding to the examples provided in the Hammond Application, many labs have published their own results showing that they successfully applied the teachings of the Hammond

Application to induce gene silencing in cultured mammalian cells using vectors that "express"[1] hairpin RNA constructs. My research group, to illustrate, has achieved gene attenuation in a wide variety of mammalian cell lines using transcribed hairpin RNAs following the teachings of the application. For instance, we have obtained gene silencing in mammalian cell lines including 293T, HeLa, COS-1, NIH 3T3, and IMR90. In each case, an expression encoding a hairpin RNA was introduced into the cultured cells and resulted in gene attenuation, as taught in the specification of the Hammond Application. No more than routine experimentation was required to select a target sequence in a mammalian gene to be silenced, design the hairpin motif that should include that sequence, generate an expression vector for transcribing the hairpin in the target mammalian cell, and than transfecting the cells with the expression vector and assessing whether or not the vector results in down-regulation of the level of expression of that gene, such as by reduced mRNA levels. There is no step beyond the teachings of the Hammond Application that involves any thing more than routine molecular biology. In fact, hairpin RNA constructs are routinely designed and generated by graduate students in my laboratory at Cold Spring Harbor, and as described below, have been used to generate more hairpin constructs against more than 125,000 (One Hundred Twenty Five Thousand) genes in a period of about one year.

4. The papers provided in Exhibits A and B describe experiments performed under my direction. The papers describe various mammalian cell lines expressing hairpin RNAs that were produced following the teachings of the abovementioned application. These examples illustrate the applicability of transcribed hairpin RNAs to mammalian cell lines in general and therefore substantiate the allegations of the application that transcribed hairpin RNA can be used to attenuate gene expression in a wide variety of mammalian cell lines.

5. The level of skill required to produce gene attenuation in mammalian cells using transcribed hairpin RNA is relatively low, in that it can be done by a molecular biologist with substantially less than doctoral training. In my research group, the preparation of mammalian cells expressing hairpin RNAs is routinely carried out by graduate students with little more experience and training than a bachelor's degree, regardless of the choice of mammalian cell line. In particular, Patrick Paddison, Amy Caudy, and Emily Bernstein, who are listed as authors on one or both of the papers provided in Exhibits A and B, were first, second, and third year graduate students, respectively, when these experiments were conducted.

---

[1] In the context of double-stranded RNA such as hairpin RNA, as described in the Hammond Application, the term "express" is used to connote transcription (though obviously not translation) of a vector sequence corresponding to the dsRNA.

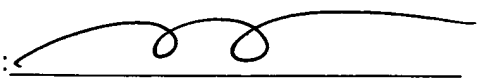
6. Exhibits C and D provide a paper and manuscript describing experiments performed under my direction. The documents describe libraries of transcribed hairpin RNAs that may be used, for example, for studying loss of function genetics in mammalian cells.

- The paper of Exhibit C describes experiments in which mammalian cells were cultured on a microarray slide spotted with different double stranded RNA constructs in a transfection carrier. Individual cell cluster become transfected with a defined double stranded RNA through a process referred to as "reverse transfection." The paper shows that this procedure was broadly applicable for achieving gene silencing using double stranded RNAs in a variety of mammalian cell types including transformed and nontransformed cell lines, fibroblast and epithelial cells, and mouse and human cells. In particular, silencing results were achieved with HEK 293T, IMR90/E1A, NIH 3T3, and HeLa cell lines. The paper further describes thirty different short hairpin RNAs (shRNAs) encoded by expression vectors that were tested in various experiments.
- The manuscript of Exhibit D describes a large scale library that we have generated which contains more than 125,000 shRNA expression plasmids covering 2/3 of all predicted genes in the human and mouse genomes. Design and construction of this very large library took only about 1 year for my laboratory to complete. The paper and manuscript of Exhibits C and D demonstrate the general applicability of transcribed hairpin RNAs for gene silencing of a wide variety of target genes in a wide variety of mammalian cells. Accordingly, these documents further substantiate the allegations of the application that transcribed hairpin RNAs are broadly applicable for gene silencing in mammalian cell lines.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Gregory Hannon

Dated: 7/28/05

Signature: 

PTO/SB/92 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application No. (if known): 09/866557

Attorney Docket No.: CSHL-P02-010

Certificate of Mailing under 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on August 9, 2005
Date

Andrea Berlo
Signature

Andrea Berlo
Typed or printed name of person signing Certificate

Registration Number, if applicable

Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Declaration Under 35 U.S.C. § 1.132 of Gregory Hannon (3 pages)
Declaration Under 35 U.S.C. § 1.132 of Gregory Hannon (7 pages)